40 MicroRNA target *CFTR* in ΔF508 CF airway epithelium

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Objectives: Expression of the cystic fibrosis transmembrane conductance regulator is altered in individuals with the DF508 CFTR mutation. MicroRNA (miRNA) are differentially expressed in the CF airway epithelium, however their role in regulation of CFTR expression here remains unexplored. Herein we investigated the role of up regulated miRNAs in CFTR regulation *in vivo* in bronchial brushings from individuals homozygous or heterozygous for DF508 CFTR, validated our observations *in vitro* and assessed the impact of defective chloride ion conductance, genotype and *Pseudomonas* status on miRNA expression.
Methods: MiRNA target prediction was performed *in silico*, expression of miRNAs and target genes was measured by qRT-PCR and/or western blotting. Modulation of miRNA was carried out using pre-miRs or antagomirs and a luciferase reporter gene employed to elucidate miRNA/CFTR interaction. MiR-145, -223 and 494 were up regulated in CF versus non-CF bronchial brushings and cell lines, in DF508 homo- vs. heterozygotes, in subjects positive for *P. aeruginosa* and in 16HBE14o– cells treated with a CFTR inhibitor. Reciprocal down or up regulation of CFTR gene and/or protein expression was observed following miRNA manipulation and direct miRNA/target relationship demonstrated via a luciferase reporter system containing the full length 3'-untranslated region of CFTR.
Conclusions: Increased expression of miR-145, -223 and -494 *in vivo* in bronchial epithelium of individuals carrying the DF508 CFTR mutation correlates with decreased CFTR expression. Defective CFTR function and *Pseudomonas* colonisation may affect miRNA expression and contribute to the regulation of DF508 CFTR mutation.

41 Corrector effect of resveratrol on refractory adrenergic pathway in saliva secretion of cystic fibrosis mice

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Objectives: Antioxidant and anti-inflammatory properties have being claimed for resveratrol, a polyphenol found in plant foods; thus we raised the hypothesis that this compound would have beneficial effects in CF.
Methods: We tested the effect of resveratrol (50 mg/kg) or vehicle, applied once-daily by intraperitoneal injection, during 4 days, on constitutive and β-adrenergic stimulated saliva secretion in F508del-CFTR (CF) and wild-type (WT) mice. A salivary secretion test was performed 2 h after resveratrol or vehicle injection on day 2 and 4. The basal, constitutive contribution of β-adrenergic pathway of saliva secretion flow (SSF; μg/min/g body weight), assessed after blocking the cholinergic pathway with local 10^{-3} M atropine, was significantly reduced in untreated CF mice compared to WT mice (median [range] SSF 4.8 [2.4–8.1] μg/min/g in female CF); values in WT mice were at least twice higher ($p = 0.037$; Wilcoxon test). As expected, β-agonist 10^{-4} M isoprenaline stimulation produced a dramatic increase in WT SSF. In contrast, no detectable change was seen in CF mice. Resveratrol treatment removed the refractory effect of CF β-agonist response by increasing SSF by at least 3-fold in female mice; a trend being observed in male mice.
Conclusion: These data confirm that β-adrenergic pathway of saliva secretion is reduced in CF mice and indicate that resveratrol rescues CFTR-dependent refractory adrenergic pathway of saliva secretion in CF mice.

42 Quantitation of CFTR protein by marker proteins

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Introduction: Only few studies have yet described immunoblot analysis of CFTR in human tissue. In the context of the upcoming studies on correctors and potentiators of mutant CFTR the quantitation of the amount of detected CFTR protein is a critical parameter to examine the function of the correctors and potentiators by immunoblot analysis. CFTR is expressed at the apical membrane of epithelial cells. CFTR immunoblot analysis should be performed in tissues that are most afflicted in CF, i.e. airways and the intestine. Of the many possible options, the analysis of rectal tissue is most suitable, because the excision of rectal tissue is safe, painless and repeatable, which is important for studies with correctors and potentiators.
As apical epithelial marker proteins for rectal biopsies sucrase-isomaltase, villin and chloride channel 2 were chosen.
Objectives: To quantify the amount of detected CFTR protein as this is the critical parameter.
Methods: The three candidates and CFTR were tested in rectal biopsies from non-CF volunteers. Signal intensity of all three proteins was reliable, but the chloride channel 2 showed cross reactivity to the used secondary antibodies. Villin and sucrase-isomaltase were evaluated as useful marker proteins for the quantitation of CFTR isolated from rectal biopsies. Because the immunoblot analyses can be done from tissue, which had been examined before by ICM (intestinal current measurement), protein analyses can be combined with functional analyses of the correctors and potentiators on the same tissue specimen.
Conclusion: In conclusion, villin and sucrase-isomaltase are suited marker proteins for quantitation of CFTR immunoreactive signals.